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Grasp the Proteome®

Protein Assay

The BCA™ Protein Assay — Reducing Agent Compatible

BCA is always compatible with more detergents, buffers/salts and solvents than any other colorimetric protein assay. Now it's compatible with reducing agents at concentrations routinely used in protein sample buffers!

The BCA™ Assay† provides one of the most accurate measurements of protein concentration in biological samples available. Although the BCA™ Assay is compatible with more detergents, buffers/salts and solvents than any colorimetric protein assay, the presence of disulfide reducing agents, including dithiothreitol (DTT) and 2-mercaptoethanol interferes with the assay (Figure 1). The BCA™ Protein Assay Kit – Reducing Agent Compatible† (Product # 23250) from Pierce provides all the advantages of the original BCA™ Assay as well as compatibility with reducing agents at concentrations routinely used in protein sample buffers (Figures 1 and 3).

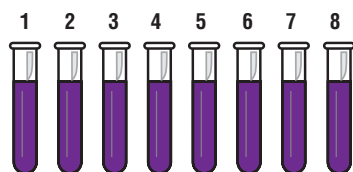


Figure 1A
Standard BCA™ Protein Assay

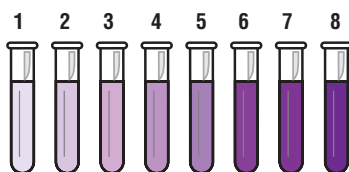


Figure 1B
BCA™ Protein Assay – Reducing Agent Compatible

Figure 1. Stylized comparison of reducing agent compatibility of standard BCA™ Protein Assay with the BCA™ Protein Assay – Reducing Agent Compatible. Reducing agents (> 1 mM) interfere with the standard BCA™ Protein Assay by artificially increasing the color intensity (Figure 1A). The color intensity produced by the BCA™ Protein Assay – Reducing Agent Compatible is unaffected by the presence of reducing agents (Figure 1B). All samples contained 5 mM DTT and BSA standards at the following concentrations: Tube 1: 0 µg/ml, Tube 2: 125 µg/ml, Tube 3: 250 µg/ml, Tube 4: 500 µg/ml, Tube 5: 750 µg/ml, Tube 6: 1,000 µg/ml, Tube 7: 1,500 µg/ml and Tube 8: 2,000 µg/ml.

Step 1. Eliminate reducing agent interference.

Step 2. Add 1 ml BCA™ Working Reagent.

Step 3. Cool sample 5-10 minutes at room temperature.

Step 4. Read at 562 nm.

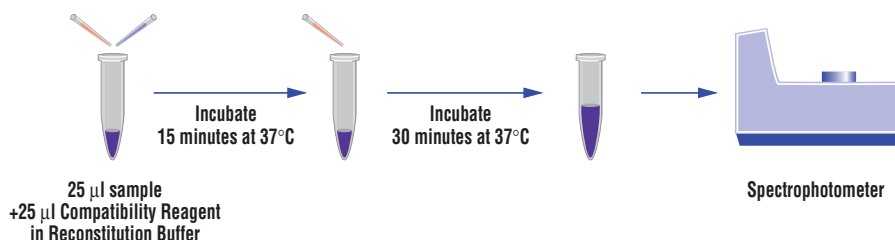


Figure 2. BCA™ Protein Assay – Reducing Agent Compatible protocol.

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Protein Assay Compatibility Table

Overview of substances compatible with Pierce Protein Assays*

Substance	BCA – Reducing Agent Compatible	BCA™ Assay	Micro BCA™ Assay	Modified Lowry	Coomassie (Bradford)	Coomassie Plus – The Better Bradford™ Assay
Detergents	(<)	(<)	(<)	(<)	(<)	(<)
Tween®-20	10%	5%	5%	0.062%	0.062%	0.031%
Triton® X-114	2%	1%	0.05%	0.031%	0.125%	0.062%
Triton® X-100	10%	5%	5%	0.031%	0.125%	0.062%
CHAPS	10%	5%	1%	0.062%	5%	5%
SDS	10%	5%	5%	1%	0.125%	0.016%
Octyl β-thioglucopyranoside	10%	5%	5%	n/a	3%	3%
Zwittergent 3-14	2%	1%	n/c	n/a	0.025%	0.025%
Reducing Agents						
Dithiothreitol (DTT)	5 mM	1 mM	n/c	n/c	5 mM	5 mM
2-Mercaptoethanol	35 mM	1 mM	1 mM	1 mM	1 M	1 M
TCEP	10 mM	n/c	n/c	n/a	n/a	n/a
Chelating Agents						
EDTA	20 mM	10 mM	0.5 mM	1 mM	100 mM	100 mM
Sodium Citrate	100 mM	200 mM	5 mM	0.1 mM	200 mM	200 mM
Buffers/Salts						
Tris	50 mM	100 mM	50 mM	10 mM	2 M	2 M
HEPES, pH 7.5	200 mM	100 mM	100 mM	1 mM	100 mM	100 mM
MES, pH 6.1	100 mM	100 mM	100 mM	125 mM	100 mM	100 mM
Imidazole, pH 7.0	50 mM	50 mM	12.5 mM	25 mM	200 mM	200 mM
Guanidine•HCl	2 M	4 M	4 M	n/a	3.5 M	3.5 M
Urea	4 M	3 M	3 M	3 M	3 M	3 M
Sucrose	40%	40%	4%	7.5%	10%	10%

n/a: not assayed

n/c: not compatible

*Refer to product instruction books for more comprehensive compatibility information

Highlights:

- Compatible with up to 5 mM DTT, 35 mM 2-mercaptoethanol or 10 mM TCEP
- Linear working range: 125-2,000 µg/ml
- Sample volume: 25 µl
- Compatible with most ionic and nonionic detergents
- Significantly less (14-23%) protein:protein variation than coomassie (Bradford)-based methods
- Colorimetric method; measure at 562 nm
- Easy-to-use protocol (Figure 2)

Reference

- Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal. Biochem.* **150**, 76-85.

Ordering Information

PRODUCT #	DESCRIPTION	PKG. SIZE
23250	BCA™ Protein Assay Kit – Reducing Agent Compatible Sufficient reagents to perform 250 standard tube assays. Includes: BCA™ Reagent A, 250 ml BCA™ Reagent B, 25 ml Compatibility Reagent, 10 x 20 mg Reconstitution Buffer, 15 ml Albumin Standard (2 mg/ml), 10 x 1 ml ampules	Kit

† BCA™ Technology is protected by U.S. patent # 4,839,295.

Patent pending on Reducing Agent-compatible BCA™ Technology.

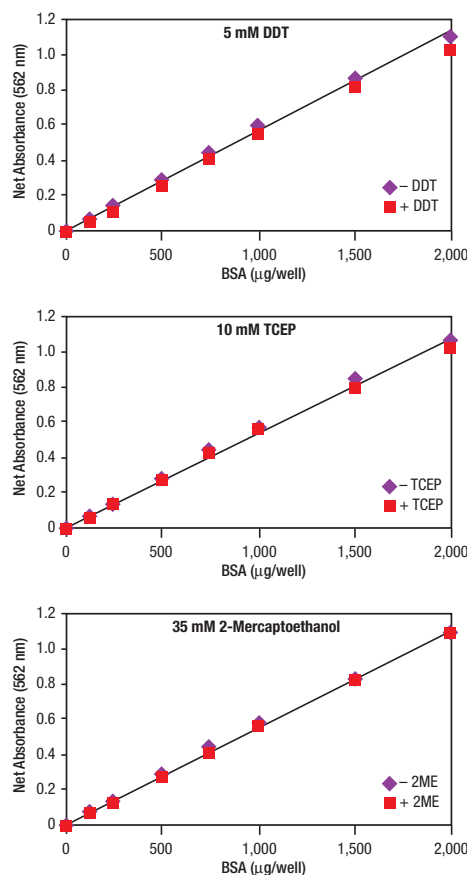


Figure 3. BCA™ Protein Assay – Reducing Agent Compatible produces a linear standard curve in the presence of reducing agents. Color response curves for BSA after treatment with Reducing Agent Compatible Reagent in the presence and absence of 5 mM DTT, 35 mM 2-mercaptoethanol and 10 mM TCEP.